REMARKS

Status of the claims

With the above amendments, claims 3 and 6-12 are amended. Claims 1-12 are pending and ready for further action on the merits. No new matter has been added by way of the above amendments. The claims are merely amended to make them clearer and to address issues that were discussed in the interview of October 13, 2004. All of the above amendments are non-narrowing in scope. Reconsideration is respectfully requested in light of the following remarks.

Rejections under 35 USC §112, second paragraph

Claims 3 and 6-12 are rejected under 35 USC §112, second paragraph as allegedly being indefinite.

Claim 3 has been rejected for reciting "said patient" with insufficient antecedent basis. Applicants have amended claim 3 to address this issue. Applicants believe that with this amendment that the rejection has been obviated. Withdrawal of the rejection is warranted and respectfully requested.

Claim 6 is rejected for referring to a PCT publication. Claim 6 has been amended so that it no longer contains this

reference. Applicants believe that with this amendment that the rejection has been obviated. Withdrawal of the rejection is warranted and respectfully requested.

Claim 8 has been rejected because the Examiner asserts that it is not known if it is the packaging material or the pharmaceutical agent that is pharmaceutically active. Applicants have amended claim 8 so that it unambiguously recites that it is the pharmaceutical agent that is pharmaceutically active. Applicants believe that with this amendment that the rejection has been obviated. Withdrawal of the rejection is warranted and respectfully requested.

Claim 8 is also rejected for the language "a combination of the before said". Applicants have omitted this language so this rejection is moot. Applicants believe that with this amendment that the rejection has been obviated. Withdrawal of the rejection is warranted and respectfully requested.

Claim 9 is rejected for reciting "a compound of the class of cyclosporins". Applicants have omitted this language so this rejection is moot. Applicants respectfully submit that the Examiner and the Examiner's supervisor objected to this claim as not further limiting the claim from which it depends (i.e., claim 8). It is believe that the amendment also obviates this

objection. Thus, Applicants believe that with this amendment that both the rejection and objection have been obviated. Withdrawal of the rejection is warranted and respectfully requested.

Claims 11 and 12 have been rejected because the Examiner asserts that they are improperly worded Jepson claims. Applicants have amended these claims so that they no longer recite that they are "an improved method", and thus, they are no longer even remotely close to being in Jepson form. Applicants believe that with this amendment that the rejection has been obviated. Withdrawal of the rejection is warranted and respectfully requested.

Rejections under 35 USC §112, first paragraph

Claims 3-12 are rejected under 35 USC §112, first paragraph as allegedly not being enabled.

The Examiner asserts that the claims are not enabled for the various methods of administration of the cyclosporin composition. Applicants traverse. Applicants assert that one of skill in the art would be able to administer the composition as claimed in the claims by any of the recited methods of administration. Applicants assert that these methods are all

well-known in the art so that the skilled practitioner could administer this composition without undue experimentation. The Examiner is reminded that "a patent need not teach, and preferably omits, what is well known in the art." *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81, 94 (Fed. Cir. 1986) and Spectra-Physics Inc. v. Coherent Inc., 3 USPQ2d 1737, (Fed. Cir. 1987). For this reason alone, Applicants submit that the rejection is inapposite. Withdrawal of the rejection is warranted and respectfully requested.

In the Examiner's Interview Summary from the October 13, 2004 Interview, the Examiner and the Examiner's supervisor requested references showing that the instant method claims are enabled for the full scope of the diseases that the cyclosporin composition purportedly can treat. Applicants, herein, provide a list of references and their teachings showing that the instant claims are enabled for the diseases claimed. What follows is a synopsis of those references and their teachings.

The following references show that cyclosporin is a known neuroprotectant, i.e., proof that neuronal damage could be prevented or reduced by cyclosporin. A search was done on PubMed Abstracts of scientific articles from February 1999 and before and the following references were produced. Please note

that the original filing date of the instant application is February 1999.

United States Patent
5,972,924

Keep, et al.
October 26, 1999
Treatment of cerebral ischemia and cerebral damage with neuroprotective agents

Abstract
The invention provides pharmaceutical compositions and medications useful for the treatment of cerebral ischernia, cerebral insult, and cerebral disorders using the active treatment medication ***cyclosporin*** *A, variants or pharmaceutically acceptable derivatives thereof, inconditions, situations and methods wherein the blood-brain barrier has been opened, disrupted, bypassed, transgressed, obviated or crossed,such that* ***cyclosporin*** *A, variants or pharmaceutically acceptable derivatives thereof come into contact with neurons and neuron support cells. Included in the invention are methods for the use of the said pharmaceutical compositions and medications. Also included in the invention are conditions, situations and methods whereby the active treatment medication can come in contact with neurons and neuron support cells.*

Thus, it is believed that cyclosporin can "prevent" neuronal damage, as well as "reduce" neuronal damage depending on the severity of the insult.

Regarding Claim 11, the enumerated diseases are:

Alzheimer's disease: *There are several February 1999 and prior abstracts (from PubMed http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) below, which show that cyclosporin could be useful in this disease. In addition, the inventors' own first cyclosporin patent from 1995 mentions Alzheimer's as a disease treatable with cyclosporin (see above). The other references are:*

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J Neurosci. 1999 Feb 1;19(3):940-7.

Prostaglandin E2 stimulates amyloid precursor protein gene expression: inhibition by immunosuppressants.

Lee RK, Knapp S, Wurtman RJ.

Division of Health Sciences and Technology, Massachusetts Institute of Technology-Harvard University, Cambridge, Massachusetts 02139, USA.

Amyloid plaques that accumulate in the brains of patients with Alzheimer's disease (AD) are primarily composed of aggregates of amyloid peptides that are derived from the amyloid precursor protein (APP). Overexpression of APP in cell cultures increases the formation of amyloidogenic peptides and causes neurodegeneration and cognitive dysfunction in transgenic mice. We now report that activation of prostaglandin E2 (PGE2) receptors increases cAMP formation and stimulates overexpression of APP mRNA and holoprotein in primary cultures of cortical astrocytes. Levels of glial fibrillary acidic protein were also increased by PGE2 treatment, suggesting that these cultured astrocytes resemble reactive astrocytes found in vivo. The stimulation by PGE2 of APP synthesis was mimicked or blocked by activators or inhibitors, respectively, of protein kinase A. Actinomycin D or cycloheximide also inhibited the increase in APP holoprotein stimulated by PGE2. Treatment of astrocytes with 8-Bromo-cAMP or forskolin for 24 hr also stimulated APP overexpression in

cultured astrocytes. The immunosuppressants cyclosporin A and FK-506 inhibited the increase in APP mRNA and holoprotein levels caused by PGE2 or by other treatments that elevated cellular cAMP levels; the inhibitory effect of FK-506 but not of cyclosporin A was attenuated by rapamycin. These results suggest that prostaglandins produced by brain injury or inflammation can activate APP transcription in astrocytes and that immunosuppressants may be used to prevent APP overexpression and possibly the pathophysiological processes underlying AD.

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(The below abstract is applicable to both Alzheimer's and Parkinson's disease for cyclosporin.)

Ann Neurol. 1998 Sep;44(3 Suppl 1):S134-41.

Mitochondria in neurodegenerative apoptosis: an opportunity for therapy?

Tatton WG, Chalmers-Redman RM.

Department of Neurology, Mount Sinai School of Medicine, New York, NY 10029-6574, USA.

Apoptotic cell death has been shown to constitute the terminal process in some neurodegenerative diseases, notably Alzheimer's disease and Parkinson's disease (PD). A decrease in mitochondrial membrane potential (delta psiM) causing opening of the permeability transition pore (PTP) in mitochondrial membranes has been implicated as a critical effector of apoptosis in a variety of non-neural cells. Opening of the PTP leads to the release of so-called apoptosis initiation factors that induce the degradative events of apoptosis, such as nuclear chromatin condensation and DNA fragmentation. We have extended those findings to a neuronal model of apoptosis caused by trophic withdrawal, by showing that a decrease in delta psiM is an early event occurring 2 to 6 hours before the degradative events of apoptosis. A deficiency in mitochondrial complex I activity has been demonstrated

in the substantial nigra of postmortem brains and several peripheral tissues obtained from PD patients. Because delta psiM is generated by the pumping of protons out across the inner mitochondrial membrane at the mitochondrial complexes, particularly complex I, we hypothesized that the decrease in complex activity could result in a decrease in delta psiM that would render PD substantial nigra neurons vulnerable to apoptosis. In preliminary studies, we have found a decrease in delta psiM in fibroblasts obtained from some PD patients. If a decrease in delta psiM consequent on decreased complex activity is an intrinsic defect in some PD patients, it would open a number of new avenues for the reduction of neuronal apoptosis in PD. The oncoprotein BCL-2 and the scavenger protein SOD-1 have been shown to reduce apoptosis by facilitating closure of the PTP. A number of agents have been shown to maintain BCL-2 and/or SOD-1 synthesis in damaged nerve cells and thereby reduce apoptosis. Other agents, such as cyclosporin A and some benzodiazepine receptor-binding agents, have been found to act directly on the PTP to reduce apoptosis. Accordingly, agents that maintain delta psiM and PTP closure may offer new and effective means of treating neurodegenerative apoptosis.

Biochem Biophys Res Commun. 1998 Jul 9;248(1):168-73.

Cyclosporin A increases resting mitochondrial membrane potential in SY5Y cells and reverses the depressed mitochondrial membrane potential of Alzheimer's disease cybrids.

Cassarino DS, Swerdlow RH, Parks JK, Parker WD Jr, Bennett JP Jr.

Medical Scientist Training Program, University of Virginia Health Sciences Center, Charlottesville 22908, USA.

Alzheimer's disease (AD) brains exhibit oxidative stress and a biochemical defect of complex IV

(cytochrome oxidase, COX) of the mitochondrial electron transport chain (ETC). This defect can be transferred through mitochondrial DNA (mtDNA) into clonal SY5Y cells depleted of their mtDNA. The resulting cytoplasmic hybrids or "cybrids" retain the complex IV defect and exhibit oxidative stress. We measured the mitochondrial membrane potential (delta psi m) in AD and control cybrids via H3-tetraphenylphosphonium ion (H3-TPP+) accumulation. AD cybrids exhibited a significant (about 30%) decrease in H3-TPP+ accumulation relative to controls. Acute treatment of normal SY5Ys with azide, a COX inhibitor, moderately decreased H3-TPP+ retention and strongly inhibited COX activity in a dose-dependent manner. As the mitochondrial transition pore (MTP) can be activated by reactive oxygen species and ETC inhibitors, and its opening causes delta psi m dissipation, we tested the effects of the MTP inhibitor cyclosporin A (CsA) on TPP+ accumulation. 5mM CsA increased basal H3-TPP+ accumulation in SY5Y cells about 10-fold, corresponding to about a 2-fold increase in delta psi m. In the AD cybrids, CsA increased the apparent delta psi m to the same final levels as it did in controls. These results indicate that low-conductance MTP activity contributes significantly to resting delta psi m in SY5Y cells. We propose the novel hypothesis that the COX defect and resulting oxidative stress in AD may pathologically activate the MTP, resulting in lower delta psi m and the release of mitochondrial factors involved in apoptosis.

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J Neurosci. 1998 Jun 15;18(12):4439-50.

Increased sensitivity to mitochondrial toxin-induced apoptosis in neural cells expressing mutant presenilin-1 is linked to perturbed calcium homeostasis and enhanced oxyradical production.

Keller JN, Guo Q, Holtsberg FW, Bruce-Keller AJ, Mattson MP.

Molecular and Cellular Biology Group, Department of Biology, University of Kentucky, Lexington, Kentucky 40536, USA.

Many cases of autosomal dominant early onset Alzheimer's disease (AD) result from mutations in the gene encoding presenilin-1 (PS-1). PS-1 is an integral membrane protein expressed ubiquitously in neurons throughout the brain in which it is located primarily in endoplasmic reticulum (ER). Although the pathogenic mechanism of PS-1 mutations is unknown, recent findings suggest that PS mutations render neurons vulnerable to apoptosis. Because increasing evidence indicates that mitochondrial alterations contribute to neuronal death in AD, we tested the hypothesis that PS-1 mutations sensitize neurons to mitochondrial failure. PC12 cell lines expressing a PS-1 mutation (L286V) exhibited increased sensitivity to apoptosis induced by 3-nitropropionic acid (3-NP) and malonate, inhibitors of succinate dehydrogenase, compared with control cell lines and lines overexpressing wild-type PS-1. The apoptosis-enhancing action of mutant PS-1 was prevented by antioxidants (propyl gallate and glutathione), zVAD-fmk, and cyclosporin A, indicating requirements of reactive oxygen species (ROS), caspases, and mitochondrial permeability transition in the cell death process. 3-NP induced a rapid elevation of [Ca2+]i, which was followed by caspase activation, accumulation of ROS, and decreases in mitochondrial reducing potential and transmembrane potential in cells expressing mutant PS-1. The calcium chelator BAPTA AM and agents that block calcium release from ER and influx through voltage-dependent channels prevented mitochondrial ROS accumulation and membrane depolarization and apoptosis. Our data suggest that by perturbing subcellular calcium homeostasis presenilin mutations sensitize neurons to mitochondria-based forms of apoptosis that involve oxidative stress.

Parkinson's disease: *There are several February 1999 and prior abstracts (from PubMed http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) below which show that cyclosporin could be useful in this disease. In addition the inventors' own first cyclosporin patent from 1995 mentions Parkinson's as a disease treatable with cyclosporin.*

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(Also see Tatton et al. 1998 in Alzheimer's above.)

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Biochim Biophys Acta. 1999 Jan 6;1453(1):49-62.

The parkinsonian neurotoxin MPP+ opens the mitochondrial permeability transition pore and releases cytochrome c in isolated mitochondria via an oxidative mechanism.

Cassarino DS, Parks JK, Parker WD Jr, Bennett JP Jr.

Neuroscience Program, University of Virginia Health Sciences Center, Charlottesville 22908, USA.

The mitochondrial transition pore (MTP) is implicated as a mediator of cell injury and death in many situations. The MTP opens in response to stimuli including reactive oxygen species and inhibition of the electron transport chain. Sporadic Parkinson's disease (PD) is characterized by oxidative stress and specifically involves a defect in complex I of the electron transport chain. To explore the possible involvement of the MTP in PD models, we tested the effects of the complex I inhibitor and apoptosis-inducing toxin N-methyl-4-phenylpyridinium (MPP+) on cyclosporin A (CsA)-sensitive mitochondrial swelling and release of cytochrome c. In the presence of Ca2+ and Pi, MPP+ induced a permeability transition in both liver and brain mitochondria. MPP+ also caused release of cytochrome c from liver mitochondria. Rotenone, a classic non-competitive complex I inhibitor, completely inhibited MPP(+)-induced swelling and

release of cytochrome c. The MPP(+)-induced permeability transition was synergistic with nitric oxide and the adenine nucleotide translocator inhibitor atractyloside, and additive with phenyl arsine oxide cross-linking of dithiol residues. MPP(+)-induced pore opening and cytochrome c release were blocked by CsA, the Ca2+ uniporter inhibitor ruthenium red, the hydrophobic disulfide reagent N-ethylmaleimide, butacaine, and the free radical scavenging enzymes catalase and superoxide dismutase. MPP+ neurotoxicity may derive from not only its inhibition of complex I and consequent ATP depletion, but also from its ability to open the MTP and to release mitochondrial factors including Ca2+ and cytochrome c known to be involved in apoptosis.

Brain Res. 1998 Oct 26;809(1):12-7.

Cyclosporin inhibition of apoptosis induced by mitochondrial complex I toxins.

Seaton TA, Cooper JM, Schapira AH.

University Department of Clinical Neurosciences, Royal Free Hospital School of Medicine, London, NW3 2PF, UK.

The cause of dopaminergic cell death in Parkinson's disease (PD) remains unknown, but may involve oxidative stress and mitochondrial complex I deficiency. Opening of the permeability transition pore and disruption of the mitochondrial transmembrane potential are known to be common events in the apoptotic pathway. Cyclosporin A and its non-immunosuppressant analogue, N-methyl-4-valine cyclosporin inhibit the opening of the mitochondrial megachannel. Complex I inhibitors, including MPP+, are known to induce both apoptosis in cell culture and parkinsonism in man and other primates. The present study using propidium iodide and FITC-TUNEL staining to identify apoptotic cells, demonstrates that rotenone, MPP+ and tetrahydroisoquinoline induce apoptosis in PC12 cells. Apoptosis induced by these

agents was decreased by cyclosporin A and N-methyl-4-valine cyclosporin. Thus, apoptosis induced by inhibitors of mitochondrial complex I is probably mediated by permeability pore opening and collapse of the mitochondrial membrane potential. This observation may allow the development of novel neuroprotective strategies in disorders that may involve mitochondrial dysfunction and apoptotic cell death. Copyright 1998 Elsevier Science B.V.

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Amyotrophic lateral sclerosis: *There are several February 1999 and prior abstracts (from PubMed http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) below which show that cyclosporin could be useful in this disease. In addition the inventors' own first cyclosporin patent from 1995 mentions neurodegenerative diseases as treatable with cyclosporin.*

Arch Neurol. 1988 Apr;45(4):381-6.

A double-blind study of the effectiveness of cyclosporine in amyotrophic lateral sclerosis.

Appel SH, Stewart SS, Appel V, Harati Y, Mietlowski W, Weiss W, Belendiuk GW.

Department of Neurology, Baylor College of Medicine, Houston, TX 77030.

In a double-blind placebo-controlled trial of cyclosporine in amyotrophic lateral sclerosis, no differences were observed in the monthly rate of progression or the relative risk of progression in comparing 38 patients randomized to the placebo group and 36 patients randomized to the cyclosporine group. In comparing three subgroups of patients, cyclosporine appeared to benefit men who entered the study within 18 months of the onset of first symptoms, whereas it was of no value to women or to men who entered later

than 18 months. For the men with recent onset of disease, the relative risk of progression was 0.403; the monthly rate of progression was 5.2 +/- 1.1 points with placebo and 3.5 +/- 0.7 points with cyclosporine. These provocative results support the need for a full study of cyclosporine in men with recent onset of disease.

JAMA. 1989 Jul 7;262(1):53-6.

Effects of cyclosporine therapy on plasma lipoprotein levels.

Ballantyne CM, Podet EJ, Patsch WP, Harati Y, Appel V, Gotto AM Jr, Young JB.

Section of Atherosclerosis, Baylor College of Medicine, Houston, Tex.

Accelerated atherosclerosis is a leading cause of death in long-term survivors of heart and renal transplantation and may be exacerbated by the frequent occurrence of posttransplant hyperlipidemia. Attempts to define the mechanism for hyperlipidemia in transplant recipients are confounded by dramatic changes in metabolism and nutritional status after transplantation, as well as by treatment with multiple immunosuppressive and antihypertensive drugs. To avoid these pitfalls and to determine if cyclosporine alone adversely affects lipid levels, we measured lipoprotein levels in a prospective, double-blind, randomized, placebo-controlled trial of cyclosporine in 36 men with amyotrophic lateral sclerosis. Plasma total cholesterol, triglyceride, high-density lipoprotein cholesterol, and apolipoprotein B levels were measured at baseline, 2 weeks, 1 month, and 2 months. Significant increases of 21% in total cholesterol, 31% in low-density lipoprotein cholesterol, and 12% in apolipoprotein B levels occurred only in the cyclosporine group. Cyclosporine therapy alone adversely affects plasma lipoprotein

levels by increasing total cholesterol levels, primarily due to an increase in low-density lipoprotein cholesterol level.

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APMIS. 1992 Jan;100(1):81-6.

Detection of nephrotoxicity in cyclosporine-A amyotrophic lateral sclerosis patients by means of urinary cytology.

Pecorella I, Ciardi A, Monge A, Bragoni M, Di Tondo U.

Dipartimento di Biopatologia Umana, University of Rome, La Sapienza, Italy.

Exfoliative urinary cytology was used for two amyotrophic lateral sclerosis patients treated with 3 mg/kg/day cyclosporine (CsA) therapy in order to detect the onset of the nephrotoxic side-effects of the drug before the apparent deterioration of the patients' clinical condition. Of the two patients, only one showed clear morphological features of drug-related damage in a one year course of cyclosporine therapy, but these followed the increase in the serum kidney and liver laboratory parameters and did not prove useful for the early detection of nephrotoxicity. However, in this patient the renal damage was hallmarked by an increasing number of tubular cells or clusters of ill-defined renal cells in the urinary specimen, suggesting an ongoing tubular injury. The slight cytological alterations may possibly be due to the low CsA dosage used for these patients. The significance of these observations is tempered by the limited number of patients and specimens studied to date and further studies in nontransplanted patients are therefore advocated, particularly when higher CsA doses are employed.

Multiple sclerosis: *There are several February 1999 and prior abstracts (from PubMed http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) below which show that cyclosporin could be useful in this disease.*

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J Neuroimaging. 1997 Jan;7(1):1-7.

Clinical and magnetic resonance imaging changes correlate in a clinical trial monitoring cyclosporine therapy for multiple sclerosis. The MS Study Group.

Zhao GJ, Li DK, Wolinsky JS, Koopmans RA, Mietlowski W, Redekop WK, Riddehough A, Cover K, Paty DW.

Division of Neurology, University of British Columbia, Vancouver Hospital and Health Sciences Centre, Canada.

Magnetic resonance imaging (MRI) was used to monitor cyclosporine therapy for chronic progressive multiple sclerosis in a multicenter clinical trial and an analysis was performed to determine whether there was a correlation between clinical changes and MRI changes. MRI was performed on 163 patients at the onset and completion of the 2-year study. Burden of disease (BOD, lesion load) was quantitated by a single observer using a computer program. Active lesions were also identified. The Expanded Disability Status Scale (EDSS) score was determined every 3 months MRI data did not show any effect of cyclosporine treatment on BOD progression (mean 24.5% increase/yr) or lesion activity. However, there was a statistically significant positive correlation between the baseline total BOD value and the baseline EDSS score (r = 0.221, p = 0.005) and a positive correlation between the percent changes in BOD from baseline to exit and EDSS score (r = 0.186, p = 0.018). The study supports the concepts that MRI is a useful technique in monitoring therapeutic trials and that MRI is a direct measure of pathology.

Optom Clin. 1996;5(3-4):193-203.

What is your diagnosis? Multiple sclerosis.

Mathews DE.

VitreoRetinal Foundation, Memphis, Tennessee, USA.

Multiple sclerosis is a demyelinating disease of the central nervous system that commonly presents with ocular manifestations. These ocular manifestations include vision loss and optomotor deficit. Treatment modalities should include treatment of the systemic disease as well as the ocular disease. New treatment protocol suggests that the best way to treat optic neuritis is with IV methylprednisolone, unless there is some serious contraindication for the patient. The use of interferon and cyclosporin as well as other anti-inflammatory agents may be useful in the future.

J Periodontol. 1994 Aug;65(8):744-9.

Gingival overgrowth in cyclosporine A treated multiple sclerosis patients.

Hefti AF, Eshenaur AE, Hassell TM, Stone C.

Department of Periodontology, University of Florida College of Dentistry, Gainesville.

Correlations have been reported between cyclosporine A (CsA)-induced gingival overgrowth (OG) and plaque-induced gingivitis, duration of CsA therapy, and blood and tissue drug levels. We evaluated the relative importance of such factors using data from a 2-year, double-blind study of CsA therapy in multiple sclerosis (MS) patients. Ninety subjects (40 taking CsA; 50 placebo) were evaluated for plaque, calculus, gingivitis, probing depths, attachment levels, and CsA levels in blood and saliva. OG was determined by a panel of 11 calibrated examiners from standardized

clinical photographs taken at the end of the study. Logistic regression was used to determine which factors were associated with occurrence of OG. Four (17%) out of 23 CsA patients with CsA trough blood levels < 400 ng/ml exhibited OG. In contrast, 10 (59%) out of 17 CsA patients with CsA trough blood levels > or = 400 ng/ml were affected with OG. Logistic regression analysis resulted in odds ratios of 0.74 (P = 0.009), 17.3 (P = 0.024) and 10.1 (P = 0.030) for the associations between OG and age, CsA trough blood levels > or = 400 ng/ml, and the interaction "color x tone," respectively. In conclusion, the incidence of CsA induced OG appears to be higher with CsA trough blood levels greater than 400 ng/ml.

Ann Neurol. 1994;36 Suppl:S108-12.

Design strategies in multiple sclerosis clinical trials. The Cyclosporine Multiple Sclerosis Study Group.

Ellison GW, Myers LW, Leake BD, Mickey MR, Ke D, Syndulko K, Tourtellotte WW.

Department of Neurology, School of Medicine, University of California, Los Angeles.

After analyzing our natural history data on the course of multiple sclerosis (MS) in more than 500 patients followed for 20 years and our experience in several therapeutic trials, we concluded that a phase III (full) trial for efficacy should have certain properties. For a power of 0.8, alpha of 0.05, and attrition rate of 10% per year, we think the trial should have a minimum sample size of 130 (65 in each arm; placebo versus active) if the design is based upon the proportion of subjects worsening by clinical measures. No stratification by entry Extended Disability Status Scale score is needed if worsening is defined as a change of 1.0 units (2 to 0.5 steps) maintained for 90 days for an entry score of 1 to 5.0 units; or 0.5 units (1 to 0.5 steps) if the entry

score is 5.5 to 7 units. We need not stratify by course (relapsing-remitting versus relapsing-progressive) but are less certain about progression from the onset. No run-in period is required to define "activity." Minimum time for treatment is 3 years. We review the justification for our conclusions; modifications in sample size that are necessary if survival analysis is used; impact of the interferon-beta trial (future trials will have an "active" control); and alternative strategies possible if magnetic resonance imaging serves as the primary outcome.

J Neurol Sci. 1993 Jul;117(1-2):192-6.

Serum prolactin levels in active multiple sclerosis and during cyclosporin treatment.

Reder AT, Lowy MT.

Department of Neurology, University of Chicago, IL.

Prolactin is essential for immune function. Excess prolactin augments some immune reactions, whereas low serum levels of prolactin inhibit immune function and prevent experimental allergic encephalomyelitis, an animal model of multiple sclerosis (MS). Activated lymphocytes, characteristics of MS, release prolactin. In this study, serum prolactin levels were normal in 35 patients with chronic progressive MS and 19 patients with acute exacerbations. These results suggest it is unlikely that prolactin contributes to the enhanced immune reactivity characteristic of MS. Acute cyclosporin A (CsA) administration increases circulating prolactin levels in animals and might paradoxically augment some immune reactions. We find that chronic CsA therapy for MS does not cause elevations in serum prolactin and should not reverse any therapeutic effect of CsA. Disturbances of prolactin regulation are not characteristic of MS.

Schweiz Rundsch Med Prax. 1991 Jun 11;80(24):670-2.
Related Articles, Links

[Efficacy and tolerance of cyclosporin A in the treatment of multiple sclerosis]

[Article in French]

Schluep M, Steck AJ, Despland PA, Regli F, Ochsner F, Berrut E, Gauthier G.

Service de neurologie, CHUV, Lausanne.

The use and tolerance of cyclosporine A (Cy A) and azathioprine (AZA) are compared as long-term immunosuppression treatment for multiple sclerosis. 38 patients with multiple sclerosis were randomized and received either Cy A (5 mg/kg/d) or AZA (2 mg/kg/d) during 24 months. These patients were assessed clinically and with different biological parameters. The Cy A blood level was controlled, and Cy A was well tolerated without side effects forcing to stop the treatment. However, its benefits were limited when the different clinical scores are compared.

Acta Neurol Scand. 1991 Jan;83(1):52-4.

Treatment of acute exacerbations in early multiple sclerosis: cyclosporin A or prednisolone?

Ruutiainen J, Salonen R, Halonen P, Panelius M, Eskola J, Salmi A.

Department of Neurology, University of Helsinki, Finland.

Twenty-six acute exacerbations in 26 patients with early definite multiple sclerosis (MS) were treated with oral cyclosporin-A (CyA) or oral prednisolone in a double-blind, controlled and randomized trial. The duration of the treatment was 6 weeks. All of the patients showed improvement during the treatment.

There were no differences in outcome between patients on CyA (7.5 mg/kg) or prednisolone (decreasing doses from 0.8 mg/kg) during the 6 week treatment. However, the improvement of clinical signs 3 months after the treatment was slightly greater in the prednisolone group. The drugs did not have significant side-effects. There was no fluctuation in the CD4/CD8 ratio during the follow-up. The two treatment groups did not differ from each other in respect to the number of CD3 (T3), CD4 (T4), CD8 (T8), CD14 (monocytes), CD20 (B cells) or CD25 (interleukin-2 receptor positive cells). The number of active T cells with the interleukin-2 receptor was high in the beginning of the exacerbation but it decreased during the treatment. To conclude, the effects of CyA and prednisolone were comparable in the treatment of acute MS relapses.

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HIV neuropathy: *There are several February 1999 and prior abstracts (from PubMed http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) below which show that cyclosporin could be useful against "HIV" disease, and "HIV dementia and neuron loss". In addition the inventors' first cyclosporin patent from 1995 mentions "HIV neuropathy" as a disease treatable with cyclosporin.*
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Exp Neurol. 1998 Dec;154(2):276-88.

HIV-1 protein Tat induces apoptosis of hippocampal neurons by a mechanism involving caspase activation, calcium overload, and oxidative stress.

Kruman II, Nath A, Mattson MP.

Department of Anatomy and Neurobiology, University of Kentucky, Lexington, Kentucky, 40536, USA.

Patients infected with HIV-1 often exhibit cognitive deficits that are related to progressive neuronal

degeneration and cell death. The protein Tat, which is released from HIV-1-infected cells, was recently shown to be toxic toward cultured neurons. We now report that Tat induces apoptosis in cultured embryonic rat hippocampal neurons. Tat induced caspase activation, and the caspase inhibitor zVAD-fmk prevented Tat-induced neuronal death. Tat induced a progressive elevation of cytoplasmic-free calcium levels, which was followed by mitochondrial calcium uptake and generation of mitochondrial-reactive oxygen species (ROS). The intracellular calcium chelator BAPTA-AM and the inhibitor of mitochondrial calcium uptake ruthenium red protected neurons against Tat-induced apoptosis. zVAD-fmk suppressed Tat-induced increases of cytoplasmic calcium levels and mitochondrial ROS accumulation, indicating roles for caspases in the perturbed calcium homeostasis and oxidative stress induced by Tat. An inhibitor of nitric oxide synthase, and the peroxynitrite scavenger uric acid, protected neurons against Tat-induced apoptosis, indicating requirements for nitric oxide production and peroxynitrite formation in the cell death process. Finally, Tat caused a delayed and progressive mitochondrial membrane depolarization, and cyclosporin A prevented Tat-induced apoptosis, suggesting an important role for mitochondrial membrane permeability transition in Tat-induced apoptosis. Collectively, our data demonstrate that Tat can induce neuronal apoptosis by a mechanism involving disruption of calcium homeostasis, caspase activation, and mitochondrial calcium uptake and ROS accumulation. Agents that interrupt this apoptotic cascade may prove beneficial in preventing neuronal degeneration and associated dementia in AIDS patients. Copyright 1998 Academic Press.

Cell. 1998 Nov 25;95(5):595-604.

Host control of HIV-1 parasitism in T cells by the nuclear factor of activated T cells.

Kinoshita S, Chen BK, Kaneshima H, Nolan GP.

Department of Molecular Pharmacology, Stanford University School of Medicine, California 94305, USA.

Post HIV-1 entry, productive HIV-1 infection of primary T cells requires overcoming several cellular blocks to provirus establishment and replication. Activation of unknown host intracellular events overcomes such inhibitory steps and is concomitant with HIV-1 replication. We show that the transcription factor NFATc was sufficient as a cellular factor to induce a highly permissive state for HIV-1 replication in primary CD4+ T cells. NFATc overcame a blockade at reverse transcription and permitted active HIV-1 replication. Pharmacologic blockade of endogenous NFAT activity by FK506 or CsA inhibited synthesis of reverse transcription and also potently blocked HIV-1 replication. T cells therefore can become competent for HIV-1 replication by control of regulated host factors such as the NFATc transcription factor. The host mechanisms regulated by such permissivity factors are potential targets for anti-HIV-1 therapy.

Virology. 1998 Jun 5;245(2):197-202.

Cyclophilin a modulates processing of human immunodeficiency virus type 1 p55Gag: mechanism for antiviral effects of cyclosporin A.

Streblow DN, Kitabwalla M, Malkovsky M, Pauza CD.

Department of Pathology and Laboratory Medicine, University of Wisconsin, Madison 53706, USA.

The molecular chaperone cyclophilin A (Cyp A) modulates human immunodeficiency virus type 1 (HIV-1) infectivity through its interactions with Gag structural proteins. The molecular mechanism for CypA in HIV-1 replication is not known. We studied chaperone effects on Gag precursor processing using cyclosporin A (CsA) to bind CypA and prevent its interaction with p55Gag. CsA treatment inhibited

p55Gag processing in extracellular virus-like particles produced from COS cells. We confirmed the effect of CsA on Gag processing by examining virions produced from CEMx174 cells infected with HIV-1LAI. Particles accumulated in the presence of CsA displayed mostly immature virion morphology and lacked condensed capsids. CsA has a direct effect on HIV-1 Gag processing that implicates CypA as having an important role in the maturation of HIV-1 particles.

Mol Med Today. 1995 Sep;1(6):287-91.

Cyclosporins: immunosuppressive drugs with anti-HIV-1 activity.

Thali M.

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Cyclosporin A was introduced into clinical use in the late 1970s to reduce graft rejection after organ transplantation. This drug, a cyclic undecapeptide metabolite of the fungus Tolypocladium inflatum, interferes with lymphokine biosynthesis, hence its immunosuppressive activity. Recently, it has become clear that cyclosporins are also inhibitors of HIV-1 replication. Immunosuppressive and antiviral activities are distinct functions of the cyclosporins, but both functions require an interaction of the drug with cyclophilins.

Guillain-Barre syndrome: *There is one pre-Febuary 1999 abstract (from PubMed http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) below which shows that cyclosporin could be useful in this disease. In addition the inventors' own first cyclosporin patent from 1995 mentions Guillain-Barre syndrome as a disease treatable with cyclosporin.*

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Fortschr Neurol Psychiatr. 1991 May;59(5):183-9.

[Immunotherapy of chronic Guillain-BarrÃ© syndrome with high dose IgG and cyclosporin A. Case report, review of the literature and perspectives]

[Article in German]

Mullges W, Ringelstein EB, Sommer C, Biniek R, Glockner WM.

Neurologische Klinik, Klinikum der RWTH, Aachen.

We report about the three-year treatment of a patient with chronic relapsing Guillain-Barre syndrome (GBS), who, simultaneously, suffered from benign gammopathy. A casual relationship between the two diseases could not be proven, since specific antimyeline antibodies could not be found. Five severe bouts of the disease occurred during the observation period, only the first two relapses, however, showed improvement with cortisone treatment, while the latter did not so. High dosages of 7S-immunoglobuline, by contrast, led to a rapid improvement during each of the subsequent relapses. The additional therapy with ciclosporine A kept the patient free from neurological deficits for more than six months now. The mechanisms of various immunological therapeutic approaches are discussed, particularly as an alternative to plasmapheresis. Our observation, as well as theoretical considerations, suggest the aforementioned immunological treatment being promising in chronic GBS.

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Neural Transplantation and Neural Xenotransplantation: *There are several pre-Febuary 1999 abstracts (from PubMed http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) below which shows that cyclosporin could be useful in both neural transplantation, and in xenotransplantation (between species).*

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J Neurosurg. 1988 Jul;69(1):121-6.

Brain xenografts: the effect of cyclosporin A on graft survival.

Howard MA 3rd, Dacey RG Jr, Winn HR.

Department of Neurological Surgery, University of Washington School of Medicine, Seattle.

Animal models of Parkinson's disease and Alzheimer's disease have shown dramatic functional improvement after transplantation of embryonic neurons into denervated regions of the adult brain. Because of the ethical and logistic problems associated with the use of human embryonic brain tissue, cross-species transplants are an attractive alternative. An experimental model of cross-species brain transplantation was developed to evaluate cell survival in untreated and cyclosporin A (CyA)-treated animals. Cholinergic ventral neurons from embryonic mice were transplanted into the frontal lobes of 18 adult Sprague-Dawley rats using a cell suspension technique. Nine animals were treated for 13 days with CyA (10 mg/kg/day) and nine were not treated. Twelve weeks after transplantation, frozen sections through the transplant volume were obtained. Alternate sections were prepared with hematoxylin and eosin and acetylcholine esterase stains. Cell counts through a 2-cu mm volume incorporating the transplant were compared to a contralateral control volume. Eight of the nine untreated transplants were successful (mean transplant cells +/- standard error of the mean: 90.7 +/- 19.4/2 cu mm). All of the nine CyA-treated transplants survived, with mean transplant count 28.7 cells/2 cu mm greater than untreated transplants (mean increase 28.7: p less than or equal to 0.05, Wilcoxon matched-pairs signed ranks test). It is concluded that: 1) this model is useful for quantitating transplant cell survival; 2) untreated xenografts survive well; and 3) a 13-day course of CyA improved

long-term graft survival.

Cell Transplant. 1999 Jan-Feb;8(1):153-9.

Cyclosporine A-induced hyperactivity in rats: is it mediated by immunosuppression, neurotrophism, or both?

Borlongan CV, Stahl CE, Fujisaki T, Sanberg PR, Watanabe S.

National Institutes of Health, National Institute on Drug Abuse, Intramural Research Program, Cellular Neurobiology, Baltimore, MD 21224, USA. cborlong@intra.nida.nih.gov

Cyclosporine A (CsA) immunosuppressive treatment has become an adjunctive therapy in neural transplantation of dopamine-secreting cells for treatment of Parkinson's disease (PD). Recently, CsA and its analogues have been shown to promote trophic effects against neurodegenerative disorders, and therefore CsA may have direct beneficial effects on dopaminergic neurons and dopamine-mediated behaviors. The present study examined the interaction between the reported CsA-induced hyperactivity and the possible alterations in nigral tyrosine hydroxylase (TH)-immunoreactive neurons in rats with damaged blood-brain barrier. CsA was administered at a therapeutic dose (10 mg/kg/day, IP, for 9 days) used in neural transplantation protocol for PD animal models. CsA-treated animals displayed significantly higher general spontaneous locomotor activity than control animals at drug injection days 7 and 9. Histological assays at day 9 revealed that there was a significant increase in TH-immunoreactive neurons in the nigra of CsA-treated rats compared to that of the vehicle-treated rats. The nigral TH elevation was accompanied by suppressed calcium-phosphotase calcineurin activity, indicating an inhibition of host immune response. This is the first report of CsA exerting simultaneous immunosuppressive and neurotrophic effects, as well as increasing general spontaneous locomotor behavior. These results support the utility of CsA as a

therapeutic agent for PD and other movement disorders.

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Exp Brain Res. 1997 Jun;115(1):71-82.

Effects of graft pooling of foetal rat and mouse tissue and immunosuppression in the 6-hydroxydopamine rat model of Parkinson's disease.

Schwarz SC, Sauer H, Oertel WH, Earl CD, Kupsch AR.

Klinikum Grosshadern, Department of Neurology, Munich, Germany.

We employed intracerebral co-transplantation of foetal xenogeneic striatal mouse tissue and allogeneic rat substantia nigra into the adult rat brain to elucidate the effects of xenogeneic mouse graft on the function and survival of an allogeneic rat graft in 6-hydroxydopamine lesioned Sprague-Dawley rats. Foetal mouse striatum (STR) and rat substantia nigra (VM) were transplanted as non-pooled separate deposits or a pooled cell suspension with or without cyclosporin A (Cy A). Immunosuppressed recipients of pooled rat and mouse co-grafts showed a significantly better compensation of amphetamine-induced rotational behaviour compared with non-immunosuppressed animals with pooled rat and mouse co-grafts 3 and 6 weeks post-grafting.Tyrosine hydroxylase (TH) immunohistochemistry revealed a non-significant reduction in survival in pooled (1806.3+/-367.5 cells) rat and mouse co-transplants without immunosuppression compared with immunosuppressed pooled (3383.3+/-732.7 cells) animals with allo- and xenogeneic tissue and controls (3506.4+/-839.3 cells). Graft volumes were significantly reduced in pooled transplants without immunosuppression (0.1+/-0.026 mm3; ANOVA post-hoc Scheffe F-test, P<0.0001) compared with immunosuppressed recipients (0.7+/-0.1 mm3) and controls (0.6+/-0.1 mm3). In non-pooled allo- and xenogeneic grafts without immunosuppression the survival rate of the TH-immunoreactive cells and graft

volumes were reduced (2359.3+/-479.5 cells; 0.2+/-0.043 mm3) compared with immunosuppressed animals (2927.3+/-946.6 cells; 0.6+/-0.2 mm3) and controls (2701.1+/-693.8 cells; 0.3+/-0.1 mm3) without reaching a level of significance. Rejection of mouse tissue was observed in all non-immunosuppressed recipients. In summary: (i) continued immunosuppression yielded significant beneficial effects on function and beneficial effects on survival of pooled grafts with an immunogenetic disparity; (ii) the rejection of a xenogeneic graft component may compromise survival and function of other, allogeneic graft components; and (iii) transplantation of non-pooled allo- and xenogeneic tissues may result in a better survival of the graft compared with pooled cell suspensions.

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Exp Neurol. 1996 Jul;140(1):1-13.

Xenotransplantation of porcine fetal ventral mesencephalon in a rat model of Parkinson's disease: functional recovery and graft morphology.

Galpern WR, Burns LH, Deacon TW, Dinsmore J, Isacson O.

Neurogeneration Laboratory, McLean Hospital, Harvard Medical School, Massachusetts 02178, USA.

Neurotransplantation of human fetal dopamine (DA) neurons is currently being investigated as a therapeutic modality for Parkinson's disease (PD). However, the practical limitations of human fetal transplantation indicate a need for alternative methodologies. Using the 6-hydroxydopamine rat model of PD, we transplanted dopaminergic neurons derived from Embryonic Day 27 porcine fetuses into the denervated striatum of cyclosporine-A (CyA)-treated or non-CyA-treated rats. Functional recovery was assessed by amphetamine-induced rotation, and graft survival and morphology were analyzed using neuronal and glial immunostaining as well as in situ hybridization with a

porcine repeat element DNA probe. A significant, sustained reduction in amphetamine-induced rotational asymmetry was present in the CyA-treated rats whereas the non-CyA-treated rats showed a transient behavioral recovery. The degree of rotational recovery was highly correlated to the number of surviving transplanted porcine dopaminergic neurons. TH+ neuronal survival and graft volume were significantly greater in the CyA-treated group as compared to the non-CyA group. By donor-specific neuronal and glial immunostaining as well as donor-specific DNA labeling, we demonstrate that porcine fetal neuroblasts are able to survive in the adult brain of immunosuppressed rats, mediate functional recovery, and extensively reinnervate the host striatum. These findings suggest that porcine DA neurons may be a suitable alternative to the use of human fetal tissue in neurotransplantation for PD.

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N Engl J Med. 1992 Nov 26;327(22):1541-8.

Unilateral transplantation of human fetal mesencephalic tissue into the caudate nucleus of patients with Parkinson's disease.

Spencer DD, Robbins RJ, Naftolin F, Marek KL, Vollmer T, Leranth C, Roth RH, Price LH, Gjedde A, Bunney BS, et al.

Neural Transplant Program, Yale University School of Medicine, New Haven, Conn. 06510.

BACKGROUND. Parkinson's disease is characterized by the loss of midbrain dopamine neurons that innervate the caudate and the putamen. Studies in animals suggest that fetal dopaminergic neurons can survive transplantation and restore neurologic function. This report compares the clinical results in four case patients with severe Parkinson's disease who underwent stereotaxic implantation of human fetal ventral mesencephalic tissue in one caudate nucleus with the results in a control group of similar subjects

assigned at random to a one-year delay in surgery. METHODS. Each case patient received cryopreserved tissue from one fetal cadaver (gestational age, 7 to 11 weeks). Before implantation, adjacent midbrain tissue underwent microbiologic, biochemical, and viability testing. Cyclosporine was administered for six months postoperatively. RESULTS. The procedure was well tolerated. Three case patients showed bilateral improvement on motor tasks, as assessed on videotape, and were more functional in the activities of daily living, as assessed by themselves and neurologists, during both optimal drug therapy and "drug holiday" periods. One case patient, who died after four months from continued disease progression, had striatonigral degeneration at autopsy. In the patients who received transplants, optimal control was achieved with a lower dose of antiparkinsonian medications, whereas the controls required more medication. Positron-emission tomography with [18F]fluorodopa before and after surgery in one patient revealed a bilateral restoration of caudate dopamine synthesis to the range of normal controls, but continued bilateral deficits in the putamen. CONCLUSIONS. Although the case patients continued to be disabled by their disease, unilateral intracaudate grafts of fetal tissue containing dopamine diminished the symptoms and signs of parkinsonism during 18 months of evaluation.

Exp Brain Res. 1989;77(2):329-36.

Xenografting of fetal pig ventral mesencephalon corrects motor asymmetry in the rat model of Parkinson's disease.

Huffaker TK, Boss BD, Morgan AS, Neff NT, Strecker RE, Spence MS, Miao R.

Hana Biologics, Inc., Alameda, CA 94501.

A suspension of cells from embryonic day 21 fetal pig ventral mesencephalon was transplanted into the striatum of 20 immunosuppressed rats with 6-

hydroxydopamine-induced lesions of the nigrostriatal dopamine pathway. Of these rats, 15 showed reduction of amphetamine-induced ipsilateral rotation by 9 weeks and complete reversal of rotation by 14-17 weeks. Animals maintained stable reversal of rotations (contralateral direction) until cessation of Cyclosporin A (CyA) treatment at 15-20 weeks. Within 4-9 weeks after CyA removal, these rats showed exclusively ipsilateral rotations during behavioral testing which were comparable to pre-transplant levels, suggesting that the grafts were rejected upon cessation of CyA treatment. Rats were sacrificed and tyrosine hydroxylase (TH) immunohistochemistry was performed at several time points, both on and off CyA, to examine a possible correlation between the degree of rotational behavior and the number of TH-positive surviving grafted cells. Staining showed large numbers (230-12,329) of TH-positive surviving cells in animals displaying a high degree of rotational correction (1.6 to -9.6 net ipsilateral rotations/min) after cessation of CyA treatment. Two control groups, those transplanted with non-neuronal cells from the pig ventral mesencephalon (n = 5) and those receiving only daily CyA injections (n = 4) showed no significant reduction of net ipsilateral rotations throughout the experiment. No TH-positive surviving cells were seen in the one non-neuronal transplant analyzed. This data demonstrates long-term retention of xenografted tissue with immunosuppression and its concomitant restoration of normal motor behavior in the rat model of Parkinson's disease.

J Neurosurg. 1988 Jul;69(1):121-6.

Brain xenografts: the effect of cyclosporin A on graft survival.

Howard MA 3rd, Dacey RG Jr, Winn HR.

Department of Neurological Surgery, University of Washington School of Medicine, Seattle.

Animal models of Parkinson's disease and Alzheimer's disease have shown dramatic functional improvement after transplantation of embryonic neurons into denervated regions of the adult brain. Because of the ethical and logistic problems associated with the use of human embryonic brain tissue, cross-species transplants are an attractive alternative. An experimental model of cross-species brain transplantation was developed to evaluate cell survival in untreated and cyclosporin A (CyA)-treated animals. Cholinergic ventral neurons from embryonic mice were transplanted into the frontal lobes of 18 adult Sprague-Dawley rats using a cell suspension technique. Nine animals were treated for 13 days with CyA (10 mg/kg/day) and nine were not treated. Twelve weeks after transplantation, frozen sections through the transplant volume were obtained. Alternate sections were prepared with hematoxylin and eosin and acetylcholine esterase stains. Cell counts through a 2-cu mm volume incorporating the transplant were compared to a contralateral control volume. Eight of the nine untreated transplants were successful (mean transplant cells +/- standard error of the mean: 90.7 +/- 19.4/2 cu mm). All of the nine CyA-treated transplants survived, with mean transplant count 28.7 cells/2 cu mm greater than untreated transplants (mean increase 28.7: p less than or equal to 0.05, Wilcoxon matched-pairs signed ranks test). It is concluded that: 1) this model is useful for quantitating transplant cell survival; 2) untreated xenografts survive well; and 3) a 13-day course of CyA improved long-term graft survival.

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Rheumatol Int. 1997;17(3):85-90.

Xenobiotic immunosuppressive agents: therapeutic effects in animal models of autoimmune diseases.

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An unprecedented arsenal of new xenobiotic immunosuppressive agents has been developed recently. Most of the new immunosuppressants have been tested primarily in the treatment of allograft rejection in experimental models of transplantation, and some of the new drugs have already proven their safety and efficiency in extensive clinical trials on transplant patients. Another field for their potential application is the treatment of autoimmune diseases. This review will give an overview of the therapeutic potential of the new xenobiotic drugs in different animal models of rheumatoid arthritis, systemic lupus erythematosus, myasthenia gravis, multiple sclerosis, diabetes mellitus, thyroiditis and uveoretinitis. The new xenobiotics are either inhibitors of the de novo synthesis of nucleotides, for example mycophenolate mofetil, mizoribine, leflunomide, and brequinar, or are immunophilin-binding agents (cyclosporin, FK506 and rapamycin) that inhibit signal transduction and cell cycle progression in lymphocytes. A different mode of action is likely to account for the immunosuppressive effects of deoxyspergualin, which may interfere with intracellular chaperoning by the heat shock protein HSP70 and the activation of transcription factor NF-kappa B.

Exp Brain Res. 1986;65(1):235-40.

Behavioural effects of human fetal dopamine neurons grafted in a rat model of Parkinson's disease.

Brundin P, Nilsson OG, Strecker RE, Lindvall O, Astedt B, Bjorklund A.

The ventral mesencephalon, containing the developing dopaminergic neurons of the substantia nigra-ventral tegmental region, was obtained from aborted human fetuses of 9-19 weeks of gestation. The tissue was grafted into the striatum of rats previously subjected to a 6-hydroxydopamine lesion of the mesostriatal

dopamine pathway. The graft recipients were immunosuppressed by daily injections of Cyclosporin A. Amphetamine-induced motor asymmetry was reduced, and finally totally reversed, only in rats receiving grafts from the 9-week old fetal donor. The fluorescence microscopic analysis revealed large numbers of surviving dopamine neurons, and extensive fiber outgrowth into the host striatum, in these rats. By contrast, rats receiving grafts from 11-19 week old donors had at most only few surviving dopamine neurons. These results indicate that human fetal mesencephalic tissue may be an efficient source of dopamine neurons for functional intracerebral grafting in patients with Parkinson's disease.

Exp Neurol. 1998 Jan;149(1):97-108.

Safety of intrastriatal neurotransplantation for Huntington's disease patients.

Kopyov OV, Jacques S, Lieberman A, Duma CM, Eagle KS.

Neurosciences Institute, Good Samaritan Hospital, Los Angeles, California 90017, USA.

Fetal neural transplantation has been shown to be a feasible, safe, and according to a number of recent reports, effective treatment for Parkinson's disease (PD). Fetal striatal transplantation may be as feasible, safe, and effective a treatment for Huntington's disease (HD), a disorder for which there is currently no effective treatment. This report describes our experience with fetal striatal transplantation to adult striatum in three HD patients. Three moderately advanced, nondemented HD patients received transplantation of fetal striatal tissue. The striatal precursor was selectively obtained from the lateral ganglionic eminence. Each patient received bilateral grafts from five to eight donors, placed into the caudate nucleus (one graft on each side) and the putamen (four grafts on each side). All three patients had HD as documented by family history, DNA heterozygosity (17-20 and 48-51 repeats),

magnetic resonance imaging (MRI) revealing striatal atrophy, and 2-deoxyglucose positron emission tomography revealing striatal hypometabolism. All patients had been evaluated using the Unified Huntington's Disease Rating Scale and appropriate neuropsychological tests for at least 3 months prior to transplantation. One year following transplantation, MRI of all three patients revealed that the grafts survived and grew within the striatum without displacing the surrounding tissue. No patients demonstrated adverse effects of the surgery or the associated cyclosporin immunosuppression, nor did any patient exhibit deterioration following the procedure. The limited experience provided by these three patients indicates that fetal tissue transplantation can be performed in HD patients without unexpected complications.

Exp Brain Res. 1989;75(1):213-20.

A primate model of Huntington's disease: cross-species implantation of striatal precursor cells to the excitotoxically lesioned baboon caudate-putamen.

Isacson O, Riche D, Hantraye P, Sofroniew MV, Maziere M.

Department of Anatomy, University of Cambridge, U.K.

Ibotenic acid was injected unilaterally into the baboon caudate-putamen (CP) to achieve a neural degeneration model in the primate, with a neuropathology similar to Huntington's disease. Four to six weeks later injections of cell suspensions of striatal precursor cells, obtained by dissection of the fetal rat striatal region (13-15 days gestational age), were made into the excitotoxically lesioned CP of 3 baboons immunosuppressed by Cyclosporin A. Morphological analysis indicated that in one of the baboons, which had the largest lesion of the CP and the shortest survival time (6 weeks after implantation), there was a surviving striatal implant.

The implanted neurons grew in high densities in cellular aggregates within the host gliotic CP. These neurons had a neuronal size phenotypical for rat striatum, i.e. on average about a 25% smaller neuronal cell diameter than a similar population in the baboon caudate-putamen. Glial-fibrillary-acid-protein immunoreactivity was present on large astrocytes within the striatal implant, with a distinct border towards the lesion-induced astrogliosis of the host. Neuronal markers for acetylcholinesterase and Leu-enkephalin were distributed in a typical patchy manner in the striatal implants along with fiber staining for tyrosine-hydroxylase-like immunoreactivity (TH) possibly derived from afferent host dopaminergic axons. Some of these fibers in the implants came from intrinsic TH-positive neuronal somata, probably of neocortical fetal origin and transiently expressing the enzyme. In conclusion, the results indicate that neuronal replacement can be achieved by cross-species implantation of fetal striatal precursor cells to the previously neuron depleted primate CP under immunosuppression but that the survival and growth of such implants may be variable and subject to unfavourable trophic conditions.

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Stroke: *There are numerous pre-Febuary 1999 abstracts (from PubMed http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) below which shows that cyclosporin could be useful in this disease. In addition the inventors' own first cyclosporin patent from 1995 mentions stroke (ischemic brain damage) as a disease treatable with cyclosporin.*

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Neurochem Res. 1999 Jan;24(1):9-13.

Early treatment with cyclosporin A ameliorates the reduction of muscarinic acetylcholine receptors in gerbil hippocampus after transient forebrain ischemia.

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Recent evidence has suggested that cyclosporin A (CsA), an immunosuppressive agent, has neuroprotective properties. However, its mechanisms associated with this activity remain unclear. We have previously shown that post-ischemic administration of CsA daily for 14 days prevented the decrease of muscarinic acetylcholine receptor binding in the hippocampus in the gerbil model of 5-min transient forebrain ischemia. In the present study, CsA (5 mg/kg, subcutaneously) was administered to each animal just after, 2 and 6 h after ischemia so as not to exert its immunosuppressive effect. Initial CsA treatment significantly restored the declined muscarinic acetylcholine receptor binding of the hippocampus 14 days after ischemia similar to the previous report. However, CsA did not alter reactive changes of astrocytes and microglia in the CA1 area of the hippocampus, which had been suppressed by daily administration. These results indicate that CsA could positively modulate the hippocampal acetylcholine neurotransmission system broken down through the ischemia-induced pyramidal cell death and its action mechanism may have no relation to the immunosuppressive properties.

Brain Res. 1998 Nov 23;812(1-2):216-26.

Amelioration by cyclosporin A of brain damage in transient forebrain ischemia in the rat.

Uchino H, Elmer E, Uchino K, Li PA, He QP, Smith ML, Siesjo BK.

Department of Anesthesiology, Tokyo Medical College, 6-7-1 Nishishinjuku Shinjuku-ku 160, Tokyo, Japan.

The immunosuppressant drug cyclosporin A (CsA) is considered to be inherently protective in conditions of ischemia, e.g. in hepatic and cardiac tissue. However, investigations of effects of CsA on neuronal tissue have been contradictory, probably because the blood-brain barrier (BBB) is virtually impermeable to CsA. In the present study, we exploited the finding that the insertion of a syringe needle into brain parenchyma obviously disrupts the BBB and allows influx of CsA, and explored whether CsA, given as intraperitoneal injections daily for 1 week before and 1 week after forebrain ischemia of 7 or 10 min duration, ameliorates the damage incurred to the hippocampal CA 1 sector. In other experiments, the needle insertion and the first i.p. injection of CsA were made 30 min after the start of recirculation, with continued daily administration of CsA during the postinsult week. In animals which were injected with CsA in daily doses of 10 mg kg-1, but in which no needle was inserted, the drug failed to ameliorate CA1 damage, whether the ischemia had a duration of 7 or 10 min. Likewise, needle insertion had no effect on CA1 damage if CsA was not administered. In contrast, when CsA was given to animals with a needle insertion, CA1 damage was dramatically ameliorated, whether treatment was initiated 1 week before ischemia, or 30 min after the start of recirculation. The effect of CsA seemed larger than that of any other drug proposed to have an anti-ischemic effect in forebrain/global ischemia. Injection of tritiated CsA in one animal with BBB disruption lead to detectable radioactivity throughout the ventricular system, suggesting a generalised increase of the entry of CsA across the BBB. The results demonstrate that immunosuppressants of the type represented by CsA markedly ameliorate delayed neuronal damage after transient forebrain ischemia, provided that they can pass the BBB. It is discussed whether the effect of the drug is one involving calcineurin, a protein phosphatase, or if CsA counteracts a permeability transition of the inner mitochondrial membrane, assumed to occur in response to adverse conditions, e.g. gradual accumulation of

Ca2+ in the mitochondria in the postischemic period. Copyright 1998 Elsevier Science B.V.

Stroke. 1998 Mar;29(3):705-18.

Calcium in ischemic cell death.

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BACKGROUND: This review article deals with the role of calcium in ischemic cell death. A calcium-related mechanism was proposed more than two decades ago to explain cell necrosis incurred in cardiac ischemia and muscular dystrophy. In fact, an excitotoxic hypothesis was advanced to explain the acetylcholine-related death of muscle end plates. A similar hypothesis was proposed to explain selective neuronal damage in the brain in ischemia, hypoglycemic coma, and status epilepticus. SUMMARY OF REVIEW: The original concepts encompass the hypothesis that cell damage in ischemia-reperfusion is due to enhanced activity of phospholipases and proteases, leading to release of free fatty acids and their breakdown products and to degradation of cytoskeletal proteins. It is equally clear that a coupling exists between influx of calcium into cells and their production of reactive oxygen species, such as .O2, H2O2, and .OH. Recent results have underscored the role of calcium in ischemic cell death. A coupling has been demonstrated among glutamate release, calcium influx, and enhanced production of reactive metabolites such as .O2-, .OH, and nitric oxide. It has become equally clear that the combination of .O2- and nitric oxide can yield peroxynitrate, a metabolite with potentially devastating effects. The mitochondria have again come into the focus of interest. This is because certain conditions, notably mitochondrial calcium accumulation and oxidative stress, can trigger the assembly

(opening) of a high-conductance pore in the inner mitochondrial membrane. The mitochondrial permeability transition (MPT) pore leads to a collapse of the electrochemical potential for H+, thereby arresting ATP production and triggering production of reactive oxygen species. The occurrence of an MPT in vivo is suggested by the dramatic anti-ischemic effect of cyclosporin A, a virtually specific blocker of the MPT in vitro in transient forebrain ischemia. However, cyclosporin A has limited effect on the cell damage incurred as a result of 2 hours of focal cerebral ischemia, suggesting that factors other than MPT play a role. It is discussed whether this could reflect the operation of phospholipase A2 activity and degradation of the lipid skeleton of the inner mitochondrial membrane. CONCLUSIONS: Calcium is one of the triggers involved in ischemic cell death, whatever the mechanism.

Brain Res. 1997 Apr 4;753(1):133-40.

Amelioration by cyclosporin A of brain damage following 5 or 10 min of ischemia in rats subjected to preischemic hyperglycemia.

Li PA, Uchino H, Elmer E, Siesjo BK.

Department of Anaesthesiology, Tokyo Medical College, Japan.

It has recently been shown that the immunosuppressant cyclosporin A (CsA) dramatically ameliorates the selective neuronal necrosis which results from 10 min of forebrain ischemia in rats. Since CsA is a virtually specific blocker of the mitochondrial permeability transition (MPT) pore which is assembled under adverse conditions, such as mitochondrial calcium accumulation and oxidative stress, the results suggest that the delayed neuronal death is due to an MPT. In the present study we explored whether CsA can also ameliorate the aggravated brain damage which is observed in hyperglycemic subjects, and which

encompasses rapidly evolving neuronal lesions, edema, and postischemic seizures. Anaesthetised rats with a plasma glucose concentration of approximately 13 mM were subjected to 10 min of forebrain ischemia, and allowed a recovery period of 7 days. In these animals, CsA prevented seizure from occurring and virtually eliminated neuronal necrosis. In order to allow even higher plasma glucose values (approximately 20 mM) to be studied, with long-term recovery, the duration of ischemia had to be reduced to 5 min. Again, CsA suppressed seizure activity and reduced neuronal damage. However, the effects were not as marked or consistent as in the 10 min group, suggesting that excessive tissue acidosis recruits mechanisms of damage which are not sensitive to CsA.

Acta Physiol Scand. 1995 Dec;155(4):469-71.

Cyclosporin A dramatically ameliorates CA1 hippocampal damage following transient forebrain ischaemia in the rat.

Uchino H, Elmer E, Uchino K, Lindvall O, Siesjo BK.

Laboratory for Experimental Brain Research, University Hospital, Lund, Sweden.

(No Abstract on PubMed, but full article available from me if needed)

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***Brain hemorrhage**: The inventors' own first cyclosporin patent from 1995 mentions brain hemorrhage as a disease treatable with cyclosporin.*

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Brain trauma: *There are Abstracts from February 1999 and before (from PubMed http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) below which show that cyclosporin could be useful in this disease. In addition the inventors' own first cyclosporin patent from 1995 mentions brain trauma as a disease treatable with cyclosporin.*

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Neuroreport. 1999 Feb 5;10(2):353-8.

Cyclosporin A limits calcium-induced axonal damage following traumatic brain injury.

Okonkwo DO, Buki A, Siman R, Povlishock JT.

Department of Anatomy, Medical College of Virginia, Virginia Commonwealth University, Richmond 23298-0709, USA.

In traumatic axonal injury, Ca2+ influx across a focally damaged axolemma precipitates local mitochondrial failure, degradation of the subaxolemmal spectrin network and compaction of neurofilaments, which collectively contribute to axonal failure. In previous studies, cyclosporin A pretreatment preserved mitochondrial integrity and attenuated axonal failure following trauma. Here we investigate whether this CsA-linked protection was related to the concomitant blunting of intra-axonal, Ca2+-induced cytoskeletal changes in traumatic axonal injury, assessed with antibodies targeting spectrin proteolysis and neurofilament compaction. CsA pretreatment dramatically reduced Ca2+-induced cytoskeletal damage following injury; CsA-treated rats, compared with vehicle-treated rats, displayed a 70% decrease in immunoreactive/damaged profiles. We suggest that CsA-mediated preservation of mitochondrial integrity enables the restoration of ionic and metabolic homeostasis thereby short-circuiting Ca2+-induced proteolysis in injured axons.

J Neurosurg Anesthesiol. 1998 Apr;10(2):101-5.

A case of severe cerebral trauma in a patient under chronic treatment with cyclosporine A.

Gogarten W, Van Aken H, Moskopp D, Roos N, Schleef J, Marcus M, Meyer J.

Klinik und Poliklinik fur Anesthesiologie und operative Intensivmedizin, Westfelische Wilhems-Universitet, Munster, Germany.

A case of severe cerebral head injury in a child, chronically treated with cyclosporine A after orthotopic liver transplantation, is presented. The initial Glasgow Coma Scale score after the motor vehicle accident was 3, and computed tomography showed multiple sites of intracerebral bleeding, an epidural hematoma, and signs of perifocal edema. Although these lesions are normally associated with a poor outcome, the child recovered unexpectedly well. In brain injury, a lucid interval can be followed by secondary neurologic deterioration due to a loss of high-energy metabolites, a release of neurotransmitters, and an increase in intracellular Ca2+. These events finally led to cell damage in the penumbra of an ischemic infarction. Among other drugs, immunosuppressants such as cyclosporine A have been shown to exhibit neuroprotective properties in experimental models if given during this time interval of secondary neurologic deterioration. Although human data on these effects are still lacking, we conclude that neuroprotective actions of cyclosporine A may have been involved in the favorable outcome in this 14-year-old boy.

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Spine trauma: *There is one pre-Febuary 1999 abstract (from PubMed http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) below which shows that cyclosporin could be useful in this disease. In addition the inventors' own first cyclosporin patent from 1995 mentions spinal cord injury syndrome as a disease treatable with cyclosporin.*

*****

J Neurotrauma. 1996 May;13(5):267-72.

Alteration of cyclosporin-A pharmacokinetics after experimental spinal cord injury.

Ibarra A, Guizar-Sahagun G, Correa D, Kretschmer R, Grijalva I, Flores-Murrieta FJ, Castaneda-Hernandez G, Odor A, Lopez RM, Franco-Bourland R, Espitia AL, Salgado-Ceballos H, Madrazo I.

Proyecto CAMINA A.C., Mexico, D.F., Mexico.

The pharmacokinetics of the immunosuppressive agent cyclosporin-A (CsA) were studied in rats submitted to spinal cord (SC) injury. A single CsA 10 mg/kg dose was given either intraperitoneally (i.p.) or orally to rats submitted to experimental SC injury at the T8 level. Twenty four hours after lesion (acute stage of SC injury) i.p. CsA bioavailability was increased, while t1/2 was prolonged. However, oral bioavailability was reduced. Seven weeks after lesion (chronic stage of SC injury) CsA bioavailability, by either route, was not significantly different from control values. Results indicate that parenteral CsA bioavailability is increased during the acute stage of SC lesion, probably due to an impaired elimination. Oral bioavailability, however, is decreased, since there is also an important reduction in gastrointestinal CsA absorption that overrides the effect of impaired elimination. Alterations in CsA pharmacokinetics appear to revert during the chronic stage of SC injury. Changes in CsA bioavailability,

depending on the route of administration and on time, must be considered to design an adequate immunosuppressive treatment in SC injury.

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J Neurotrauma. 1996 Oct;13(10):569-72.

Use of cyclosporin-A in experimental spinal cord injury: design of a dosing strategy to maintain therapeutic levels.

Ibarra A, Reyes J, Martinez S, Correa D, Guizar-Sahagun G, Grijalva I, Castaneda-Hernandez G, Flores-Murrieta FJ, Franco-Bourland R, Madrazo I.

Proyecto CAMINA, A.C., Mexico, D.F.

Cyclosporin-A (CsA) is frequently used as an immunosuppressive agent in experimental transplantations. CsA has been used in nervous tissue transplants in spinal cord injury (SCI). However, optimal results have not been obtained. This is likely due to the fact that SCI alters CsA pharmacokinetics and hence fixed dose regimens are not adequate. In this study, several CsA dosing regimens were evaluated in Long-Evans female rats subjected to a severe low thoracic (T8) SCI by the contusion method. Serum CsA concentrations were measured to determine which dosing regimen allowed CsA levels to be maintained within the therapeutic window. It was found that administration of 2.5 mg/kg/12 h intraperitoneally during the first 2 days after SCI (acute phase) followed by 5 mg/kg/12 h orally thereafter (subacute and chronic phases) yields CsA circulating levels within the therapeutic window, i.e., 0.120-0.275 microgram/mL. This dosing regimen represents a suitable alternative to fixed dosing to achieve an optimal CsA-induced immunosuppression in experimental models of SCI.

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Ionizing radiation: The inventors' own second cyclosporin patent from 1998 concerns the use of cyclosporin to prevent damage from ionizing radiation. This earlier patent pending preceding the February 1999 filing should make ionizing radiation allowable.

United States Patent Application 20040147433

Kind Code A1

Keep, Marcus; et al.
July 29, 2004
Neuroimmunophilins for selective neuronal radioprotection

Abstract

Method for selectively reducing mammal neuron damage or death in neuroimmunophilin-rich neurons of central, peripheral, and autonomic nervous systems of a mammal while not reducing damage or death to neuroimmunophilin-poor cells and tissues selected from the group consisting of glia, glia-derived tumor cells, abnormal neuron-derived tumor cells, non-brain tumors, and non-neuron tissue of the body from ionizing radiation. The method includes preparing a dosage of a neuroimmunophilin ligand selected from the group consisting of cyclosporins and functional derivatives, metabolites, variants, and salts thereof which are able to cross the blood-brain barrier. The dosage is from an effective amount to less than 1 gr/kg of body weight of said mammal. The method includes the step of administering that dosage to the mammal before, co-incident with, or after ionizing radiation of the mammal. The dose is administered the same day as, but not later than one week after, last radiation exposure.

Inventors:
Keep, Marcus; *(Honolulu, HI)* ***; Elmer, Eskil;*** *(Lund, SE)*

Filed:
January 15, 2004

What is claimed is:

1. A method for improved radiation treatment by selectively reducing mammal neuron death from ionizing radiation in cyclophilin-rich neurons of central, peripheral, and autonomic nervous systems of a mammal while not reducing damage or death to cyclophilin-poor cells and tissues selected from the group consisting of brain tumors, meningiomas, pituitary tumors, craniopharyngioma, lung tumors, renal tumors, breast tumors, colon tumors, skin tumors, squamous cell tumors, laryngeal tumors, and prostate tumors, said method comprising the steps of: (a) preparing a dosage of cyclophilin ligand for parenteral or enteral administration, said cyclophilin ligand being selected from the group consisting of cyclosporins and functional derivatives, metabolites, variants, and salts thereof selected from the group consisting of ***cyclosporin*** *A,* ***cyclosporin*** *C,* ***cyclosporin*** *D,* ***cyclosporin*** *G,* ***cyclosporin*** *AM1,* ***cyclosporin*** *AM9,* ***cyclosporin*** *AM1c,* ***cyclosporin*** *AM4N,* ***cyclosporin*** *AM19,* ***cyclosporin*** *AM1c9,* ***cyclosporin*** *AM1A,* ***cyclosporin*** *AM1A4N,* ***cyclosporin*** *AM1Ac,* ***cyclosporin*** *AM1AL,* ***cyclosporin*** *AM11d,* ***cyclosporin*** *AM69,* ***cyclosporin*** *AM4N9,* ***cyclosporin*** *AM14N,* ***cyclosporin*** *AM14N9,* ***cyclosporin*** *4N69,* ***cyclosporin*** *AM99N, dihydrocyclosporin CsA, dihydrocyclosporin CsC, dihydrocyclosporin CsD, dihydrocyclosporin CsG,* ***cyclosporin*** *M17,* ***cyclosporin*** *AM1c-GLC,* ***cyclosporin*** *sulfate conjugate,* ***cyclosporin*** *BH11a,* ***cyclosporin*** *BH15a,* ***cyclosporin*** *B,* ***cyclosporin*** *G,* ***cyclosporin*** *E,* ***cyclosporin*** *M1 through* ***cyclosporin*** *M26,* ***cyclosporin*** *MUNDFI,* ***cyclosporin*** *MeBMT,* ***cyclosporin*** *GM1,* ***cyclosporin*** *GM9,* ***cyclosporin*** *GM4N,* ***cyclosporin*** *GM1c,* ***cyclosporin*** *GM1c9,* ***cyclosporin*** *GM19,* ***cyclosporin*** *SDZ-209-313,* ***cyclosporin*** *SDZ-205-549,*

cyclosporin *SDZ-033-243,* ***cyclosporin*** *SDZ-IMM-125, and* ***cyclosporin*** *SDZ-PSC-833, which are able to cross the blood-brain barrier, said dosage being from 0.001 to 50 mg/kg of body weight of said mammal for parenteral administration and from 0.01 to 60 mg/kg of body weight of said mammal for enteral administration; and (b) administering said dosage to said mammal before administering ionizing radiation treatment to said mammal.*

2. An improved method in accordance with claim 1, for ionizing radiation treatment of a patient with a disease of a condition requiring ionizing radiation treatment, employing a selective neuronal ionizing protector, said method comprising: treating said patient with an effective amount of cyclophilin ligand as the selective neuronal ionizing radiation protector, said cyclophilin ligand being selected from the group consisting of cyclosporins and functional derivatives, metabolites, variants, and salts thereof, which are able to cross the blood-brain barrier.

3. The method of claim 1, wherein said ionizing radiation comprises a radiation which is selected from the group consisting of alpha radiation, beta radiation, X radiation, gamma radiation, cosmic radiation, fast neutron radiation, proton radiation, and particle beam radiation.

4. The method of claim 1, wherein said ionizing radiation exposure is therapeutic treatment radiation from medical sources, or non-therapeutic radiation from industrial sources, natural sources, man-made sources, or nuclear sources.

5. The method of claim 1, wherein said cyclophilin ligand is administered by parenteral injection, said injection being into, or adjacent to, the brain, tumor, or spinal cord, or via cerebrospinal fluid spaces, intraventricular fluid spaces, or intrathecal spaces, or via application into the digestive, respiratory, or genito-urinary systems, or skin, or

by a combination of these routes, so that said cyclophilin ligand comes into contact with neurons.

6. The method of claim 1, wherein said mammal is a cancer patient with a primary brain tumor.

7. The method of claim 1, wherein said mammal is a cancer patient with a metastatic brain tumor.

8. The method of claim 1, wherein said mammal is a patient with an ionizing radiation-treatable lesion.

9. The method of claim 1, wherein said ***cyclosporin is cyclosporin*** *A or a derivative, metabolite of salt thereof.*

10. The method claim 9, wherein said ***cyclosporin is cyclosporin*** *A.*

11. A method for selectively reducing mammal neuron death from ionizing radiation in cyclophilin-rich neurons of central, peripheral, and autonomic nervous systems of a mammal while not reducing damage or death to cyclophilin-poor cells and tissues selected from the group consisting of glia, glia-derived tumor cells, abnormal neuron-derived tumor cells, non-brain tumors, and non-neuron tissue of the body, said method comprising the steps of: (a) preparing a dosage of ***cyclosporin*** *A, said dosage being from an effective amount to less than 1 gr/kg of body weight of said mammal; and (b) administering said dosage to said mammal before, co-incident with, or after ionizing radiation of said mammal, said dose being administered not later than the same day as the radiation exposure.*

12. The method of claim 11, wherein said ionizing radiation comprises a radiation which is selected from the group consisting of alpha radiation, beta radiation, X radiation, gamma radiation, cosmic radiation, fast neutron radiation, proton radiation, and particle beam radiation.

13. The method of claim 11, wherein said ionizing radiation exposure is therapeutic treatment radiation from medical sources, or non-therapeutic radiation from industrial sources, natural sources, man-made sources, or nuclear sources.

14. The method of claim 11, wherein said cyclophilin ligand is administered by parenteral injection, said injection being into or adjacent to, the brain, tumor, or spinal cord, or via cerebrospinal fluid spaces, intraventricular fluid spaces, or intrathecal spaces, or via application into the digestive, respiratory, or genito-urinary systems, or skin, or by a combination of these routes, so that said cyclophilin ligand comes into contact with neurons.

15. The method of claim 11, wherein said mammal is a cancer patient with a primary brain tumor.

16. The method of claim 11, wherein said mammal is a cancer patient with a metastatic brain tumor.

17. The method of claim 11, wherein said mammal is a patient with an ionizing radiation-treatable lesion.

Description

[0001] This application is a continuation of application Ser. No. 09/787,861, which was filed on Jun. 14, 2001. Ser. No. 09/787,861 was a U.S. national phase application of PCT/US98/20040, which was filed on Sep. 23, 1998. Applicants claim the benefits under 35 U.S.C. §120 of the filing dates of both said applications. The entire disclosure of each of said applications is hereby expressly incorporated by reference.

Neurotoxicity of vestibulocochlear structures: *There is one pre-February 1999 abstract (from PubMed http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) below which indicates that cyclosporin could be useful against neurotoxicity of cochlear structures. In addition the inventors' own first cyclosporin patent from 1995 mentions neurotoxcity and cochlear damage as a disease treatable with cyclosporin.*

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Rev Rhum Engl Ed. 1998 Jan;65(1):63-4.

Sudden cochlear hearing loss in a patient with BehÃ§et's disease.

Narvaez J, Valverde-Garcia J, Alegre-Sancho JJ, Juanola X, Clavaguera MT, Roig-Escofet D.

Department of Rheumatology, Principes de Espana Hospital, Barcelona, Spain.

A Behcet's disease patient developed sudden cochlear hearing loss during a flare of her disease. Prednisone and cyclosporin therapy was ineffective, probably because it was initiated late. Sensorineural hearing loss is a rare but underrecognized complication of various forms of vasculitis such as Wegener's granulomatosis, polyarteritis nodosa, giant cell arteritis and Behcet's disease. Its importance lies in the need for an early diagnosis, since prompt treatment with steroids and immunosuppressive agents may lead to restoration of hearing.

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Retinal detachment: *There are two pre-Febuary 1999 abstracts (from PubMed http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) below which shows that cyclosporin could be useful in this disease. In addition the inventors' own first cyclosporin patent from 1995 mentions retinal damage as a disease treatable with cyclosporin.*

*****

Acta Ophthalmol (Copenh). 1994 Oct;72(5):533-6.

Diffuse chronic retinal pigment epitheliopathy and exudative retinal detachment.

Laatikainen L.

Department of Ophthalmology, University of Oulu, Finland.

Two patients with bilateral chronic retinal pigment epitheliopathy are presented. Both patients had large areas of pigment epithelial decompensation and small pigment epithelial detachments at the posterior pole. In the macula subretinal fluid was scanty, but the first patient developed an extensive bullous retinal detachment with shifting of subretinal fluid with changes in posture in both eyes, the second patient had similar detachment in one eye. The etiology of the pigment epithelial disorder remained unknown. No inflammatory cells were found in the vitreous specimen or subretinal fluid in the first patient. Treatment with peroral corticosteroids alone or in combination with azathioprine and cyclosporin A, as well as surgery for retinal detachment in one eye, proved unsuccessful. Argon laser coagulation of the decompensated areas in the macula resulted in resorption of subretinal fluid and reattachment of the exudative detachment.

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Invest Ophthalmol Vis Sci. 1998 Jun;39(7):1227-32.

Protective effects of FK506 against glutamate-induced neurotoxicity in retinal cell culture.

Kikuchi M, Kashii S, Mandai M, Yasuyoshi H, Honda Y, Kaneda K, Akaike A.

Department of Ophthalmology and Visual Sciences,

Graduate School of Medicine, Kyoto University, Japan.

PURPOSE: To examine the effects of FK506 on glutamate neurotoxicity in cultured retinal neurons. METHODS: Experiments were performed with primary retinal cultures obtained from 17- to 19-day-old rat fetuses. To assess the effects of FK506 and other drugs on glutamate neurotoxicity, cultures were treated with a drug beginning 10 minutes before application of glutamate and continuing during the subsequent 10 minutes of glutamate exposure. The treated cells were then incubated for 1 hour in a drug-free and glutamate-free medium. After a 1-hour incubation, cell viability was quantitatively measured by the trypan blue exclusion method. RESULTS: Brief exposure to glutamate markedly decreased cell viability. FK506 protected against glutamate neurotoxicity in a dose-dependent manner. Rapamycin is a competitive inhibitor of FK506 that binds FK506 binding protein. Simultaneous application of rapamycin and FK506 negated the protective effects of FK506. Cyclosporin A, which binds and inhibits calcineurin, mimicked the protective effects of FK506. Treatment with FK506 did not affect the intracellular maximum Ca2+ concentration induced by glutamate application. Although FK506 exhibited protective action against Ca2+ ionophore-induced neurotoxicity, it had no effect on nitric oxide-induced neurotoxicity. Treatment with FK506 reduced the activity of nitric oxide synthase (NOS). CONCLUSION: FK506 protected against glutamate neurotoxicity by inhibiting NOS activity in cultured retinal neurons.

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Regarding Claim 12, the numerous autoimmune diseases treated by cyclosporin are mentioned in the enclosed abstracts. Applicants believe that prior to February 1999, it was well known that cyclosporin was useful in a very broad swath of autoimmune diseases, justifying a relatively broad claim.

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Orv Hetil. 1996 Sep 1;137(35):1921-7.

[Cyclosporin-A therapy in autoimmune diseases]

[Article in Hungarian]

Patakfalvi A.

The author surveys the action of cyclosporin, its advantages over conventional immunosuppressive drugs and its side effects. This paper lists comparative research showing autoimmune diseases in which cyclosporin has been used as well as data confined to disease causality. It touches on almost all autoimmune diseases, and details rheumatoid arthritis, lupus erythematosus disseminatus, and the treatment of kidney disease, giving a review of the ideal usage of cyclosporin during laboratory and clinical testing and the reasons for dosage modifications. In organ transplants, it is necessary to determine the serum level of cyclosporin. In autoimmun diseases cyclosporin's effectiveness and side effects cannot be determined on the basis of blood serum level. Dose modifications should be based on serum creatinine levels and on diastolic blood pressure. On the basis of the literature and on his own experience, the author emphasizes the potential for new perspectives in drug usage. This is particularly true for Sandimmun-Neoral. His group for example has been the first to use Cyclosporin A to treat Polyglandular autoimmune syndrome and Henoch Schonlein disease and has achieved excellent results.
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Artif Organs. 1997 Sep;21(9):983-8.

Cyclosporine and therapeutic plasma exchange in treatment of progressive autoimmune diseases.

Schiel R, Bambauer R, Latza R, Klinkmann J.

University of Saarland Medical School, Homburg/Saar,

Germany.

Despite treatment with intensive immunosuppressive drug regimens, the prognosis of patients suffering from severe progressive autoimmune diseases like systemic lupus erythematosus (SLE), nephrotic syndrome (NS), and Behcet's disease is poor. Side effects (infections and malignant tumors) often occur. In the present trial, 35 patients suffering from autoimmune diseases (SLE, n = 21; NS, n = 10; and Behcet's disease, n = 4) were treated for 3.7 +/- 2.0 years with 2.5 +/- 0.6 mg cyclosporine/kg body weight/day in addition to corticosteroids alone or in combination with azathioprine and/or cyclophosphamide. In active stages of the diseases with extremely high concentrations of anti-ds-DNA-antibodies, antinuclear antibodies, circulating immunocomplexes, and reduced complement concentrations, therapeutic plasma exchange (TPE) has been applied. Compared with previous treatment modalities, significantly (p < 0.05) more effective and rapid reductions of the antibodies were reached. Clinical disorders improved within 1-6 weeks. All patients reported increased performance and a better quality of life. After 1-12 months, the previously required doses of immunosuppressive drugs and the frequency of TPE could be reduced by 40-100%. After 13.4 +/- 11.8 months in 17 of 35 patients (8 with SLE, 5 with NS, 4 with Behcet's disease), cyclosporine was established as the monotherapy. No severe side effects were registered. In treating active stages of severe progressive autoimmune diseases and forms with persistent high antibody levels, the addition of TPE to conventional therapy was very effective, as observed in both clinical and laboratory parameters.

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Curr Opin Rheumatol. 1998 May;10(3):169-73.

Ann Intern Med. 1998 Jun 15;128(12 Pt 1):1021-8.
Use of cytotoxic agents and cyclosporine in the treatment of autoimmune disease. Part 1: rheumatologic

and renal diseases.

Langford CA, Klippel JH, Balow JE, James SP, Sneller MC.

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA.

When cytotoxic agents were initially introduced, their ability to disrupt nucleic acid and protein synthesis led to their effective use for the treatment of neoplastic disease. During the course of this use, however, it became apparent that these agents also suppress the immune system. This usually unwelcome effect was subsequently studied and beneficially directed toward the treatment of non-neoplastic diseases in which autoimmune mechanisms were considered important to pathogenesis. As a result of these investigations, cytotoxic agents and, more recently, cyclosporine have emerged to become an important part of the therapeutic regimen for many autoimmune diseases. Nonetheless, these medications may still cause treatment-induced illness or even death. It is therefore particularly important to weigh the benefits and risks of cytotoxic therapy when treating a non-neoplastic disease. This two-part Clinical Staff Conference reviews data on the efficacy and toxicity of cytotoxic drugs and cyclosporine in selected autoimmune diseases. Part 1 examines the manner in which these agents have been used to treat rheumatologic and renal diseases.
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Ann Intern Med. 1998 Jul 1;129(1):49-58.

Use of cytotoxic agents and cyclosporine in the treatment of autoimmune disease. Part 2: Inflammatory bowel disease, systemic vasculitis, and therapeutic toxicity.

Langford CA, Klippel JH, Balow JE, James SP, Sneller

MC.

When cytotoxic agents were introduced, their ability to disrupt nucleic acid and protein synthesis led to their effective use for the treatment of neoplastic disease. During the course of this use, however, it became apparent that these agents also suppress the immune system. This usually unwelcome effect was subsequently studied and beneficially directed toward the treatment of non-neoplastic diseases in which autoimmune mechanisms were considered important to pathogenesis. As a result of these investigations, cytotoxic agents and, more recently, cyclosporine have emerged to become an important part of the therapeutic regimen for many autoimmune diseases. Nonetheless, these medications may still cause treatment-induced illness or even death. It is therefore particularly important to weigh the benefits and risks of cytotoxic therapy when treating a non-neoplastic disease. This two-part Clinical Staff Conference reviews data on the efficacy and toxicity of cytotoxic drugs and cyclosporine in selected autoimmune diseases. In part 2, we focus on the role of these agents in treating inflammatory bowel disease and systemic vasculitis and review the toxic effects of these agents.

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Update on immunosuppressive therapy.

Singer NG, McCune WJ.

536 RB & C, Division of Pediatric Immunology Allergy and Rheumatology, Cleveland, OH 44106, USA.

In this review we summarize selected articles that have been published about immunosuppressive agents in the past year. These studies fall into three major categories: 1) use of pulse cyclophosphamide in autoimmune diseases other than systemic lupus erythematosus; 2) use of newer immunosuppressive agents such as cyclosporine and FK506 in a variety of rheumatic diseases; and 3) toxicity.

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Am J Gastroenterol. 1999 Jan;94(1):241-8.

Cyclosporine therapy in patients with steroid resistant autoimmune hepatitis.

Fernandes NF, Redeker AG, Vierling JM, Villamil FG, Fong TL.

Cedars-Sinai Medical Center, Center for Liver Disease and Transplantation, and Liver Unit, University of Southern California School of Medicine, Los Angeles 90048, USA.

Autoimmune hepatitis is a form of chronic liver disease characterized by progressive hepatocellular inflammation, which usually responds to treatment with corticosteroids. However, 10% of patients with autoimmune hepatitis are refractory to corticosteroids and develop progressive liver disease and cirrhosis. We describe five patients with autoimmune hepatitis who did not respond to conventional corticosteroids and azathioprine therapy who were then treated with cyclosporine A. Cyclosporine A was started at 2-3 mg/kg/day and induced biochemical remission in four of five patients within 3 months. One of the four responders relapsed within 1 month of discontinuing cyclosporine on two occasions. Each time, liver tests promptly normalized after reinitiation of cyclosporine. Two responders were managed with cyclosporine alone. The single patient who did not respond to cyclosporine developed progressive liver failure, underwent orthotopic liver transplantation, and subsequently died of disseminated cytomegalovirus infection. Cyclosporine was generally well tolerated and none of the patients developed renal insufficiency. These data and review of 11 cases in the literature show that cyclosporine can induce remission of liver disease in patients with autoimmune hepatitis who are refractory to corticosteroids.

Drugs. 1993 Jun;45(6):953-1040.

Cyclosporin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in immunoregulatory disorders.

Faulds D, Goa KL, Benfield P.

Adis International Limited, Auckland, New Zealand.

Cyclosporin is a lipophilic cyclic polypeptide which produces calcium-dependent, specific, reversible inhibition of transcription of interleukin-2 and several other cytokines, most notably in T helper lymphocytes. This reduces the production of a range of cytokines, inhibiting the activation and/or maturation of various cell types, including those involved in cell-mediated immunity. Thus, cyclosporin has immunosuppressive properties, and has a proven place as first line therapy in the prophylaxis and treatment of transplant rejection. Cyclosporin has also been evaluated in a large range of disorders where immunoregulatory dysfunction is a suspected or proven aetiological factor, and this is the focus of the present review. In patients with severe disease refractory to standard treatment, oral cyclosporin is an effective therapy in acute ocular Behcet's syndrome, endogenous uveitis, psoriasis, atopic dermatitis, rheumatoid arthritis, active Crohn's disease and nephrotic syndrome. Concomitant low dose corticosteroid therapy may improve response rates in some disorders. The drug can be considered as a first line therapy in patients with moderate or severe aplastic anaemia who are ineligible for bone marrow transplantation, with the additional benefit of reducing platelet alloantibody titres. It may also be of considerable therapeutic benefit in patients with primary biliary cirrhosis, particularly those with less advanced disease. Limited evidence suggests cyclosporin is effective in patients with intractable pyoderma gangrenosum, polymyositis/dermatomyositis or

severe, corticosteroid-dependent asthma. Indeed, the steroid-sparing effect of cyclosporin is a significant advantage in a number of indications. Furthermore, the drug has shown some efficacy in a wide range of other, generally uncommon disorders in which controlled clinical trials are lacking and/or are unlikely to be performed. Cyclosporin does not appear to be effective in patients with allergic contact dermatitis, multiple sclerosis or amyotrophic lateral sclerosis. It is only temporarily effective in patients with type I (insulin-dependent) diabetes mellitus and should not be used in this indication. To avoid relapse after control of active disease, patients should receive cyclosporin maintenance therapy at the lowest effective dosage. However, maintenance therapy appears to be of no benefit in patients with Crohn's disease and cyclosporin should be discontinued in these patients once active disease is controlled. Hypertrichosis, gingival hyperplasia, and neurological and gastrointestinal effects are the most common adverse events in cyclosporin recipients, but are usually mild to moderate and resolve on dosage reduction. Changes in laboratory variables indicating renal dysfunction are relatively common, although serious irreversible damage is rare.(ABSTRACT TRUNCATED AT 400 WORDS)

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Nihon Rinsho Meneki Gakkai Kaishi. 1998 Feb;21(1):20-8.

Effective treatment of autoimmune diseases with extremely low dose cyclosporine.

Sugiyama M, Sekigawa I, Tokano Y, Iida N, Hashimoto H, Hirose S.

Department of Medicine, Juntendo Izu-Nagaoka Hospital.

The dosage of cyclosporine administered in the treatment of autoimmune diseases has generally been comparable to those required in cases of

transplantation. Here we report on the successful treatment using an extremely low dose cyclosporine (1 mg/kg/day) on four patients, involving thrombocytopenia with systemic lupus erythematosus, and interstitial pneumonitis with Sjogren's syndrome, and discuss the optimal dose of cyclosporine for autoimmune-mediated manifestations.

Klin Monatsbl Augenheilkd. 1998 Sep;213(3):182-5.

[Sympathetic ophthalmia 50 years after penetrating injury. A case report]

[Article in German]

Hellmund K, Fruhauf A, Seiler T, Naumann GO.

Augenklinik im Universitatsklinikum C. G. Carus, TU Dresden.

BACKGROUND: Sympathetic ophthalmia is a rare form of autoimmune uveitis and manifests in 90% of cases within the first year after penetrating injuries or surgical interventions. PATIENTS AND METHODS: In the present case the sympathetic ophthalmia started 50 years after a penetrating injury by a shell splinter. The injured eye was amaurotic and phthitic and the sympathizing eye showed an anterior uveitis. After an initial treatment with local and systemic corticosteroids the uveitis improved. The clinical diagnosis of sympathetic ophthalmia was made after a second inflammation course with substantial visual loss and subtotal chorioidal detachment. After enucleation of the exciting eye the diagnosis was confirmed by histological examination. An immunosuppressive therapy including azathioprine and cyclosporine became necessary to control the uveitis. RESULTS: After enucleation the corticosteroid treatment was not sufficient. Additional therapy with azathioprine resulted in a recovery of the symptoms but had to be stopped because of adverse reactions. The alternative therapy by means of cyclosporine was

tolerated well, but dose reduction was difficult because of recurrences. After a 30 month lasting cyclosporine therapy the patient shows stabile results since 6 months with visual acuity of 20/30. CONCLUSIONS: The present case report demonstrates that a delayed onset of sympathetic ophthalmia 50 years after initial trauma may occur but can be controlled by an immediate, high dose immunotherapy.

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J Hepatol. 1999 Feb;30(2):222-7.

Short-term cyclosporine induces a remission of autoimmune hepatitis in children.

Alvarez F, Ciocca M, Canero-Velasco C, Ramonet M, de Davila MT, Cuarterolo M, Gonzalez T, Jara-Vega P, Camarena C, Brochu P, Drut R, Alvarez E.

Gastroenterology Unit, Hopital Sainte-Justine, Universite de Montreal, Quebec, Canada.

BACKGROUND/AIMS: The current immunosuppressive treatment of patients with autoimmune hepatitis consists of prednisone and azathioprine. High doses of prednisone used to obtain the remission of the disease are associated with serious adverse effects. To avoid harmful consequences of prednisone therapy, we proposed to treat patients with oral cyclosporine to obtain the remission of the inflammatory process. METHODS: This is a pilot, multinational, multicenter, clinical trial involving children with autoimmune hepatitis. Thirty-two children were recruited, who according to international criteria were considered as having definite autoimmune hepatitis. Cyclosporine alone was administered for 6 months, followed by combined low doses of prednisone and azathioprine for 1 month, after which cyclosporine was discontinued. Biochemical remission of the disease was established by the follow-up of serum transaminase activity levels. Growth parameters and adverse effects of the treatment were recorded. RESULTS: Two patients were

withdrawn from the study: one for non-compliance and the other for liver failure which did not improve with cyclosporine. Of the 30 remaining patients, 25 normalized alanine aminotransferase activity levels by 6 months and all the patients by 1 year of treatment. Z-scores for height showed a trend towards improvement during treatment. Adverse effects of cyclosporine were mild and disappeared during weaning off the medication. CONCLUSIONS: Cyclosporine induced the biochemical remission of the hepatic inflammatory/necrotic process in children with autoimmune hepatitis, with few and well-tolerated adverse effects.

Applicants believe that the above satisfies the request that neuronal damage could be prevented or reduced by cyclosporin.

Regarding claims 11 and 12, the Examiner's supervisor said that references should be provided showing that cyclosporin can be used in the treatment of the enumerated diseases that appear in claim 11. Applicants believe that the above references show that cyclosporin can be used to treat the enumerated diseases in claim 11.

Regarding claim 12, the Examiner's supervisor indicated that autoimmune disease is rather broad and encompasses a great many diseases. Applicants believe that the number of references above that show a great variety of autoimmune diseases can be treated with cyclosporin justifies a claim to treating autoimmune diseases.

With the above remarks and amendments, Applicants believe that the claims, as they now stand, define patentable subject matter such that passage of the instant invention to allowance is warranted. A Notice to that effect is earnestly solicited.

If any questions remain regarding the above matters, please contact the undersigned in the Washington metropolitan area at the phone number listed below.

Pursuant to the provisions of 37 C.F.R. §§ 1.17 and 1.136(a), the Applicants hereby petition for an extension of two (2) months to December 16, 2004 in which to file a reply to the Office Action. The required fee of $225.00 is enclosed herewith.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____

KM/TBS/bsh

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